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## Osteoimmunology: from mice to humans

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**Osteoimmunology: from mice to humans.**

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**Running title:** Immune system and bone

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## **Abstract**

The immune system has been recognized as one of the most important regulators of bone turnover and its de-regulation is implicated in several bone diseases as post-menopausal osteoporosis and inflammatory bone loss; recently it has been suggested that the gut microbiota may influence bone turnover by the modulation of immune system.

The study of the relationship between immune system and bone metabolism is generally indicated under the term “osteimmunology”, the vast majority of these studies have been performed in animal models, however several data have been confirmed also in humans: this review summarizes recent data on the relationship between immune system and bone with particular regard to the data confirmed in humans.

**Key words:** osteoclast; T cells; B cells, cytokines; osteoporosis, immune system, menopause, gut microbiota.

## Introduction

Bone is an active tissue that undergoes continuous remodelling, the increase in bone resorption with unbalanced bone formation leads to bone diseases characterized by bone loss. Bone turnover is due to the combined action of bone resorbing cells, osteoclasts (OCs) and bone forming cells, osteoblasts (OBs). The regulation of these two cell types is due to multiple systemic and local factors as hormones, cytokines and mechanical load. The immune system has been recognized as one of the important regulators of bone turnover and its de-regulation is implicated in several bone diseases.

The interaction between immune system and bone has been deeply studied in condition as inflammatory diseases and post-menopausal osteoporosis.<sup>1</sup> Recently it has been suggested that gut microbiota may influence bone loss through the modulation of immune system.<sup>2</sup> Several data on the interaction between immune system and bone have been generated in animal models, whereas human data are scarce, the aim of this paper is to review the current knowledge on the role of immune system in the control of bone metabolism pointing out data confirmed in humans with particular regard to inflammatory diseases, post-menopausal osteoporosis and the role of gut microbiota.

### *Inflammatory diseases, immune system and bone.*

Rheumatoid arthritis (RA) is the paradigmatic disease linking immune alteration and bone metabolism. RA is a chronic disease characterized by immune deregulation associated with articular bone erosions, regional and systemic bone loss. Bone loss in RA is multifactorial, however, at least during the early disease phases, it is due to the activation of T cells that produce pro-osteoclastogenic cytokines.

The unbalance of T helper (Th) cells sub-types in RA may be the first driver of increased osteoclastogenesis in these patients. In RA different alterations of Th sub-set have been described, in humans conflicting results have been obtained, depending on different study designs, some studies analyse T cells in peripheral blood whereas others evaluate these cells in the synovial fluids.<sup>3</sup> The increased Th1 cells may have an important role in the pathogenesis of RA, these cells are increased in the synovium.<sup>3</sup> Th1 produces several cytokines that have been related to the control of bone turnover as IFN $\gamma$ , TNF $\alpha$  and RANKL: IFN $\gamma$  has controversial effect on OCs formation and activity, it was found both to inhibit and to activate OCs;<sup>1</sup> more recently it has been suggested that IFN $\gamma$  is responsible for the ability of OCs to act as antigen presenting cells and to modulate T-cell proliferation.<sup>4</sup>

TNF $\alpha$  and RANKL are well known pro-osteoclastogenic cytokines;<sup>5</sup> TNF- $\alpha$  induces OCs formation in presence of adequate levels of RANKL,<sup>5</sup> up-regulates stromal cells production of RANKL and increases the responsiveness of OCs precursors to RANKL. RANKL plays an essential role in bone physiology by up-regulating OCs activity and formation, its role is antagonized by its decoy receptor osteoprotegerin (OPG): the RANKL, OPG ratio is the main regulator of OCs formation and activity. In RA patients RANKL levels predict the therapeutic response to anti-TNF therapy,<sup>6</sup> and denosumab, which is an anti-RANKL monoclonal antibody, blocks the effect of increased RANKL on bone loss in RA patients by reducing bone resorption.<sup>7</sup>

Th17 are increased in RA, and play a crucial pathogenetic role in the appearance of bone erosions in mice,<sup>3,8</sup> in humans Th17 are recruited within synovium, where they exert pro-inflammatory and pro-osteoclastogenic effect.<sup>9</sup> Th17 cells produce IL-17, this cytokine is increased in RA,<sup>10</sup> and plays a crucial role in inflammation and in the development of the disease; however, its mechanism of action in the development of bone loss, especially in

relation to other known key cytokines such as IL-1, TNF- $\alpha$  and RANKL remains unclear. Recently, IL-17 has been suggested to be involved in the up-regulation of OCs formation in inflammation by increasing the release of RANKL, which may synergise with IL-1 and TNF- $\alpha$ .<sup>11</sup>

T regulatory cells (Tregs) are known as crucial in maintaining the peripheral tolerance mechanism and in preventing autoimmunity, hence a decreased number of Treg or a reduced function of these cells are possible factors involved in the chronic inflammation observed in RA joint. However different studies on Tregs phenotype and function in RA patients obtained conflicting results: some studies found a decrease in Tregs in peripheral blood from RA patients;<sup>12,13</sup> whereas others do not.<sup>14,15</sup>

In experimental models of arthritis Tregs have been shown to inhibit OCs formation and activity,<sup>16</sup> these data have been confirmed by our group also in humans during periprosthetic osteolysis.<sup>17</sup>

Non-conventional T cells as  $\gamma\delta$  T cells have been suggested to play a pathogenetic role in a murine model of RA through the production of IL-17<sup>18</sup>, however this role has not been confirmed in humans<sup>19,20</sup>. In RA patients an increased number of  $\gamma\delta$  T cells have been found in the synovia, however Th17 and not IL-17 positive  $\gamma\delta$  T cells seem to drive bone erosions in humans<sup>19</sup>. A cross-talk between  $\gamma\delta$  T cells and OCs has also been investigated in vitro, in particular it has been suggested that OCs are able to recruit and activate  $\gamma\delta$  T cells through the production of TNF $\alpha$ <sup>21</sup>, on the contrary activated  $\gamma\delta$  T cells inhibit OCs formation and activity through IFN $\gamma$  production in co-cultures<sup>22</sup>. The conflicting results in animal models, humans and in vitro suggest that a clear role for  $\gamma\delta$  T cells in the control of bone remodeling is not clearly established yet.

Taken together these data suggest that both an increase of Th1 and Th17 may be responsible for increased OCs formation and bone erosions in RA, whereas a role for Tregs and  $\gamma\delta$  T cells is more controversial.

T cells interact with OCs not only through the production of cytokines, but also through a cell to cell interaction; OCs behave like antigen presenting cells (APCs), they share a common precursor with dendritic cells and macrophages. OCs express major histocompatibility complex (MHC) molecules<sup>23</sup>, and costimulatory molecules involved in the regulation of T cells answers to immune stimuli as CD80 and CD86. CD80 and CD86 are expressed on professional APCs as well as on OCs and bind CD28 that is expressed on activated T cells, T cells activation is tightly regulated by molecules that interrupt CD80/86 and CD 28 signal as CTLA-4 that is expressed on activated T cell surface. Hence CTLA-4 modulates APCs function. These molecules play an essential role also in OCs-T cells interaction as it has been brilliantly demonstrated CD80/86-deficient mice display increased osteoclast differentiation as CD80/86-deficient OCs are not inhibited by CTLA-4 or Tregs<sup>24</sup>. In humans the use of abatacept that targets CD80/86 reduces OCs formation, whereas the use of ipilimumab that blocks CTLA-4 increases OCs formation. The interaction between T cells and OCs may be a fundamental mechanism of increased osteoclastogenesis in RA.

Accordingly to a possible function of OCs as APCs, a role for auto antibodies in bone destruction in RA patients has been postulated. It has been demonstrated that OCs function is influenced by antibodies, Fc receptors, and related molecules. In particular, in RA patients a direct effect of anti-citrullinated protein antibody (ACPAs) in the control of osteoclastogenesis has been demonstrated; it is known that in more than 70% of patients with RA ACPAs are detectable. Positivity for ACPAs is a strong predictor of bone erosions in RA, and ACPAs bind to OCs and stimulate osteoclast-mediated bone resorption by increasing the production of TNF- $\alpha$ , IL-8 and RANKL<sup>25,26,27</sup>



Clinically the activation of immune system and increased inflammation in RA correlate with joint erosions, bone mineral density and vertebral fractures.<sup>28</sup>

In RA the inflammatory milieu increases OCs formation and activity both locally and systemically, several pro-inflammatory cytokines increased in RA are responsible for increased OCs formation and activity: IL-1 acts by increasing RANKL expression by bone marrow stromal cells and directly targets OCs precursors, promoting OCs differentiation in the presence of permissive levels of RANKL. The effect of TNF- $\alpha$  on osteoclastogenesis is upregulated by IL-1.<sup>6</sup> In humans it has been demonstrated that anti-TNF and anti-IL-1 monoclonal antibodies reduce bone resorption.<sup>29</sup>

IL-6 is increased in RA and even though it is not essential for bone resorption it may contribute to the increased bone resorption in RA.<sup>30</sup>

In RA and in other chronic inflammatory condition not only bone resorption, but also bone formation is affected. The increase in TNF- $\alpha$  during inflammation induces an increase in dickkopf-1 (DKK-1), that is a negative regulator of the Wnt pathway. Wnts are a family of secreted lipid-modified proteins that bind to a receptor complex comprising frizzled and the low-density lipoprotein receptor-related proteins 5 or 6 (LRP5 or LRP6). Activation of this receptor leads to induction of bone formation by OBs. DKK-1 and sclerostin (SOST) bind to and inactivate signalling from LRP5/LRP6<sup>31</sup> inhibiting bone formation. In RA it has been shown an increase in DKK-1 and SOST, this blunts OBs formation and activity and, together with the increase in bone resorption, is responsible for reduced bone mass<sup>32</sup> and of the formation of articular bone erosions, in fact DKK-1 levels are associated with an increase in the number of articular erosions independently of age, baseline radiologic features, C-reactive protein, or disease activity.<sup>33</sup>

Systemic bone loss has been correlated with inflammation also during aging and in other inflammatory diseases, the mechanisms are very similar to the ones demonstrated in RA.<sup>1</sup>

Figure 1 summarizes the role of T cells in the regulation of osteoclastogenesis in RA.

#### *Estrogen loss, immune system and bone.*

Post-menopausal osteoporosis (PMO) is the most frequent metabolic skeletal disease, it is characterized by reduced bone mineral density and micro architectural deterioration of bone with increased fracture risk. In PMO the uncoupling between OB-mediated bone formation and OC-mediated bone resorption results in bone loss. Estrogen deficiency is the main driver of post-menopausal bone loss: during estrogen depletion OCs formation and activity are increased, this increase is partially mediated through the effect of estrogen deficiency on immune system.

Data on animals and humans demonstrated that both cellular and humoral immune responses are enhanced by estrogens.<sup>1</sup> During estrogen deficiency immune response is altered and, in particular T cells, become more active and able to produce inflammatory and pro-osteoclastogenic cytokines as TNF $\alpha$  and RANKL. Despite of some inverse reports,<sup>34,35</sup> the main body of literature firmly supports the essential role of activated T cells in regulating bone loss induced by estrogen deficiency,<sup>5,36,37,38</sup> both in animal models and in humans.

My lab and others demonstrated that in PMO osteoclastogenesis occurs only in the presence of T cells and that T cells of osteoporotic patients produce more RANKL and TNF- $\alpha$ , than controls,<sup>5</sup> RANKL directly correlates with increases in bone resorption markers and inversely with serum estrogen levels.<sup>37</sup> To confirm the effect of estrogen on

cytokines production is noteworthy that hormone replacement therapy decreases the production of pro-osteoclastogenic cytokines in postmenopausal women.<sup>39</sup>

One of the key mechanisms through which estrogen deficiency induces proliferation and life-span of bone marrow T cells is the increase in antigen presentation by macrophages and dendritic cells,<sup>36</sup> thanks to a greater expression of Class II TransActivator (CIITA), a transcriptional co-activator acting on MHCII promoter, with the final effect of up-regulation of MHCII on macrophages,<sup>23,36</sup> these data have been generated in mice models, however also in humans a role for low-grade inflammation and MHC class II expression in bone loss has been suggested.<sup>40</sup> T cells activation increases after OVX also thanks to the up-regulation of CD40 ligand (CD40L) expression, the CD40/CD40L system is crucial for T-cell activation and several functions of the immune system. It promotes macrophages activation and differentiation, antibody isotype switching, and the adequate organization of immunological memory in B cells. The increase in the number of activated CD40L-expressing T cells after OVX promotes the expression of M-CSF and RANKL by stromal cells and down-regulates the production of OPG, this results in a significant increase in osteoclastogenesis.<sup>38</sup> This mechanism has been demonstrated in mice and not confirmed in humans, nevertheless some papers confirm the role of estrogen in the regulation of immune function in humans in health or different diseases.<sup>41,42</sup> In humans the effect of estrogens on immune function has been demonstrated in the ability to modulate T cells cytokines production,<sup>42,43</sup> to answer to immune stimulation,<sup>44</sup> whereas OVX increases T cells activation.<sup>5,43</sup>

Estrogen deficiency increases the number of T cells also by increasing their thymic output, it has been demonstrated in humans that after ovariectomy (OVX) the size of the thymus increases, as well as in mice, and that T cells activation is increased after OVX.<sup>43</sup>

In mice models Th17 cells have been implicated in OVX induced bone loss, these cells increased after OVX due to the up regulation of STAT3, ROR- $\gamma$ t and ROR- $\alpha$  and down regulation of Foxp3 which antagonizes Th17 cell differentiation.<sup>9</sup> In humans a recent paper founds no effect of estradiol in the secretion of IL-17 by T cells in patients affected by multiple sclerosis,<sup>42</sup> whereas another suggests that estradiol inhibited Th17 differentiation through downregulation of Ror $\gamma$ t mRNA and protein expression.<sup>45</sup> Nevertheless in these studies the effect of estradiol was evaluated in vitro on cells from patients not in menopausal period and men, thus the results cannot be extended to women in menopause. We recently demonstrated both in mice and in humans a fundamental role for Th17 also in bone loss induced by primary hyperparathyroidism: we showed that IL-17 is upregulated by primary hyperparathyroidism in humans and by continuous PTH treatment in mice and that parathyroidectomy normalizes IL-17 production.<sup>46</sup>

Also B cells have recently been directly implicated in the regulation of bone resorption, these cells are able to produce OPG<sup>47</sup> and, under certain conditions, could produce RANKL. B cell knock out mice are osteoporotic with enhanced osteoclastic bone resorption due to decreased OPG level.<sup>47</sup> B cell OPG production is up-regulated by the activation of CD40<sup>38</sup> thus T cell signaling to B cells, through CD40L and CD40 plays an important role in regulating basal OCs formation and in regulating bone homeostasis. During estrogen deficiency the up-regulation of CD40 enhances the cross talk between T and B cells.

Activated B cells express RANKL, contributing to bone resorption,<sup>48</sup> the number of RANKL-expressing B lymphocytes in the bone marrow increases after OVX,<sup>49</sup> recently

Onal and colleagues demonstrated that RANKL produced by B cells and not by T cells plays a role in OVX induced bone loss in mice<sup>50</sup> (, this paper disagree with previous reports showing a fundamental role of RANKL produced by T cells in OVX induced bone loss (1, 5, 17, 36, 47). In humans there are not convincing data on the role of B cells in post-menopausal osteoporosis, nevertheless changes in several B lymphocyte populations in patients affected by post-menopausal osteoporosis regardless to their estrogen status have been shown.<sup>51</sup> The role of B cells in the control of bone turnover has been studied in other diseases that affect bone as RA<sup>48,52</sup> and periodontal inflammation.<sup>53</sup> These studies suggest that B cells may be involved in the control of bone turnover also in humans mainly by the production of cytokines as OPG and RANKL.

Figure 2 summarizes the role of immune system in the regulation of osteoclastogenesis in PMO.

#### *Gut microbiota, immune system and bone.*

The gut microbiota (GM) is the whole of the commensal bacteria living in our intestine, these are acquired at birth and can be considered a multicellular organ that communicates with and affects its host in numerous ways. The GM composition tends to stabilization after the first 3-year after birth, in this period GM offers several antigens for the host's immune system and during this first 3 years the neonatal immune system reaches maturity under the influence of the GM and its relation with environmental factors as diet, infections, antibiotics and breastfeeding. The GM composition is extremely variable between individuals, its symbiotic relationship with the human host is useful in food digestion and in fighting pathogens.

Environmental factors as diet, antibiotic treatments and infections can change GM composition, recently it has been suggested that the altered gut colonization patterns, associated with decreasing microbial diversity, play a central role in human health and disease and are being increasingly implicated in the physiologic, immunologic, and metabolic deregulation seen in many several chronic non-communicable human diseases (NCDs). Immune homeostasis disruption is the causal mechanism of NCDs as allergy, asthma, some autoimmune diseases, cardiovascular disease, metabolic disease, and neurodegenerative disorders, these disorders are characterized by a low grade of inflammation. Although inflammation and the pathways to disease are multifactorial, GM disbiosis may play a central role in the pathogenesis of these diseases.<sup>54</sup>

Recent studies suggest that the GM may be involved also in the OVX induced bone loss through the modulation of the immune system and, in particular, of T cells activation and pro-osteoclastogenic cytokines production. The most robust data have been produced in mice models, in particular, it has been shown that mice grown up in a bacteria free environment, i.e. mice with a sterile bowel called “germ-free” mice, have alteration of immune system and are protected by OVX induced bone loss. Germ free mice have an immature gut mucosal immune systems and a reduced number of T helper cells in the spleen, and in peripheral blood, this suggests that the GM is responsible for the correct development of systemic immunity.<sup>54</sup>

Bone mass and density are increased in germ free mice due to decreased bone resorption, without alteration in bone formation.<sup>2</sup> In these animals OCs formation is decreased thanks to a reduced number of T cells and of pro-inflammatory and pro-osteoclastogenic cytokines as IL-6 and TNF $\alpha$ , these observations suggested that the effect of GM on bone turnover is mediated through the modulation of immune system by GM.<sup>2</sup> Fecal transplantation in germ-free animals with GM from mice raised in a

conventional environment leads to a normalization of bone mass, T helper cells and OCs number.

Animal data also suggested that the use of some strain of probiotics is able to prevent OVX induced bone loss<sup>55</sup> and to increase bone formation by the up-regulation of genes involved in OBs formation and activity as Sparc and BMP-2.<sup>56</sup> Also effects of prebiotics have been evaluated in animal models showing a positive effect on GM composition and subsequently on bone mass.<sup>57</sup>

According to the data obtained in mice one may speculate that an unfavorable GM composition may increase inflammation and hence favors post-menopausal bone loss, however data obtained in humans are scarce. There are no data linking a microbiome profile to the risk of post-menopausal bone loss, whereas data on possible effect of modifying GM through the use of pro and prebiotics are scarce.

Another intriguing hypothesis on the effect of GM on bone is based on direct ability of GM to influence calcium absorption, a recent post-hoc analyses on the use of *Lactobacillus reuteri* showed an increased level of serum 25OH vitamin D in healthy subjects treated with this probiotic,<sup>58</sup> as regards the use of prebiotics a recent study showed that supplementation with galactooligosaccharide in adolescent girls increased the presence of bifidobacteria in the GM, as previously shown in rat, and improved calcium absorption.<sup>59</sup> A previous study demonstrated that a mixture of short- and long-chain inulin-type fructans, given as prebiotics during adolescence as oral supplements for a one-year period, increases bone mineralization and calcium absorption.<sup>60</sup> A possible explanation of the effect of dietary or prebiotic fiber on bone metabolism is the effect of the fiber on calcium

absorption; the microbiota ferment the fiber to short-chain fatty acids (SCFAs) reducing the gut pH, thus reducing the formation of calcium phosphates and increasing the calcium absorption favoring bone. In the studies on the effect of pre-biotics calcium absorption was investigated, whereas immune phenotype and inflammation were not. The effect of SCFAs may be more complex than the simple effect on gut pH and, indirectly, on calcium absorption, in fact some studies suggest that SCFAs increased calcium transport, whereas reducing gut pH with HCl did not; thus, SCFAs may be useful to improve the gut function and health. It has also been suggested that SCFAs influence calcium absorption through signaling pathway modulation, in fact butyrate modulates calcium absorption by non-gut cells<sup>61</sup>.

These seminal studies in mice, and less clearly in humans, suggest that alteration of GM composition influences bone metabolism through different mechanisms. The most attracting hypothesis, as suggested by the findings from germ free mice, is that the GM influences bone turnover and mass by modulating the host's immune system, however other mechanisms, as influence on calcium absorption and on vitamin D synthesis, may also be involved.

## **CONCLUSIONS.**

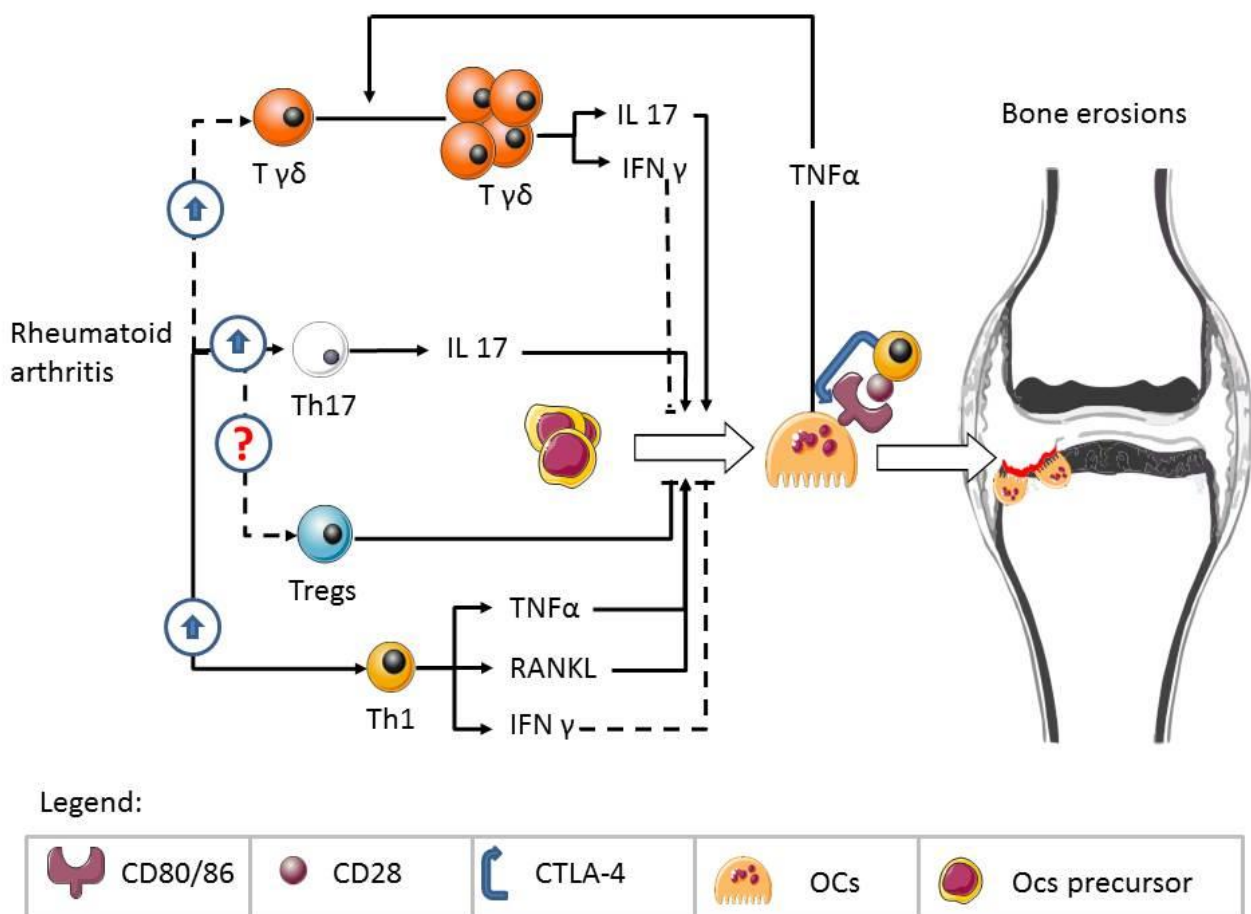
The interactions between immune system and bone are complex and play significant role in both health and disease, nevertheless not all the pathways discovered in animal models have been fully demonstrated in humans, and several challenging questions remains unsolved



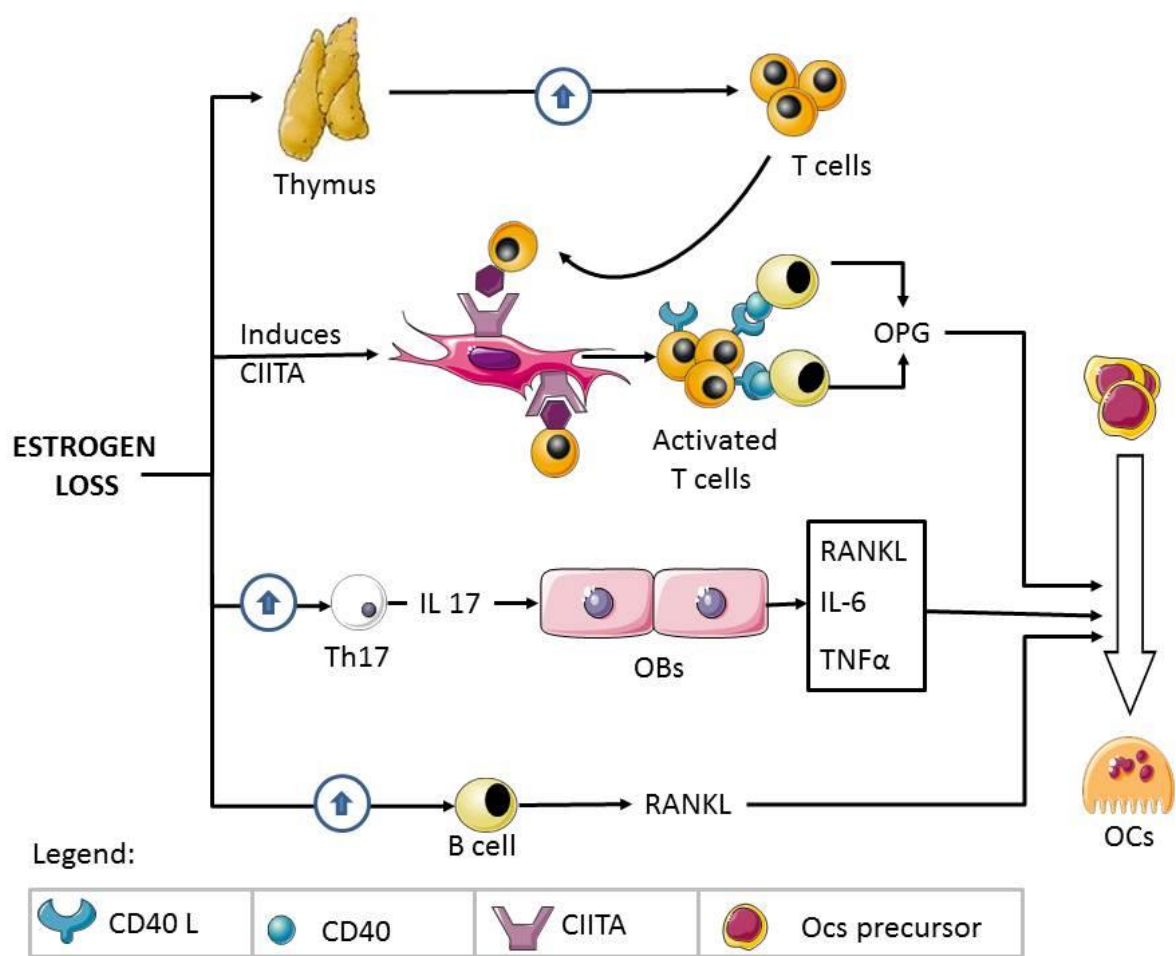
**Conflict of interest:** PD and FS have nothing to disclose.

**Figure legend.**

**Figure 1.** The cartoon represents interaction between T cells and osteoclastogenesis in RA, continuous lines represent pathways demonstrated in humans, whereas dotted lines represent pathways not clearly demonstrated in humans.



**Figure 2.** The cartoon represents interaction between estrogen deficiency, immune system and bone cells.



## REFERENCES

1. Mori G, D'Amelio P, Faccio R, Brunetti G. The interplay between the bone and the immune system. *Clin Dev Immunol* 2013; **2013**: 720504.
2. Sjögren K, Engdahl C, Henning P, Lerner UH, Tremaroli V, Lagerquist MK *et al.* The gut microbiota regulates bone mass in mice. *J Bone Miner Res* 2012; **27**: 1357-1367.
3. Komatsu N, Takayanagi H. Arthritogenic T cells in autoimmune arthritis. *Int J Biochem Cell Biol* 2015; **58**: 92-96.
4. Li H, Lu Y, Qian J, Zheng Y, Zhang M, Bi E *et al.* Human osteoclasts are inducible immunosuppressive cells in response to T cell-derived IFN- $\gamma$  and CD40 ligand in vitro. *J Bone Miner Res* 2014; **29**: 2666-2675.
5. D'Amelio P, Grimaldi A, Di Bella S, Brianza SZ, Cristofaro MA, Tamone C *et al.* Estrogen deficiency increases osteoclastogenesis up-regulating T cells activity: a key mechanism in osteoporosis. *Bone* 2008; **43**: 92-100.
6. Sakthiswary R, Das S. The effects of TNF $\alpha$  antagonist therapy on bone metabolism in rheumatoid arthritis: a systematic review. *Curr Drug Targets* 2013; **14**: 1552-1557.
7. Dore RK, Cohen SB, Lane NE, Palmer W, Shergy W, Zhou L *et al.* Denosumab RA Study Group. Effects of denosumab on bone mineral density and bone turnover in patients with rheumatoid arthritis receiving concurrent glucocorticoids or bisphosphonates. *Ann Rheum Dis* 2010; **69**: 872-875.
8. Lubberts E, Koenders MI, Oppers-Walgreen B, van den Bersselaar L, Coenen-de Roo CJ, Joosten LA *et al.* Treatment with a neutralizing anti-murine interleukin-17

- anti-body after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. *Arthritis Rheum* 2004; **50**: 650-659.
9. Tyagi AM, Srivastava K, Mansoori MN, Trivedi R, Chattopadhyay N, Singh D. Estrogen deficiency induces the differentiation of IL-17 secreting Th17 cells: a new candidate in the pathogenesis of osteoporosis. *PLoS One* 2012; **7**: e44552.
  10. Roeleveld DM, Koenders MI. The role of the Th17 cytokines IL-17 and IL-22 in Rheumatoid Arthritis pathogenesis and developments in cytokine immunotherapy. *Cytokine* 2015; **74**: 101-107.
  11. Lubberts E, Koenders MI, van den Berg WB. The role of T-cell interleukin-17 in conducting destructive arthritis: lessons from animal models. *Arthritis Res Ther* 2005; **7**: 29-37.
  12. Sempere-Ortells JM, Pérez-García V, Marín-Alberca G, Peris-Pertusa A, Benito JM, Marco FM *et al.* Quantification and phenotype of regulatory T cells in rheumatoid arthritis according to disease activity score-28. *Autoimmunity* 2009; **42**: 636-645.
  13. Zare HR, Habibagahi M, Vahdati A, Habibagahi Z. Patients with Active Rheumatoid Arthritis Have Lower Frequency of nTregs in Peripheral Blood. *Iran J Immunol* 2015; **12**: 166-175.
  14. Basdeo SA, Moran B, Cluxton D, Canavan M, McCormick J, Connolly M *et al.* Polyfunctional, Pathogenic CD161<sup>+</sup> Th17 Lineage Cells Are Resistant to Regulatory T Cell-Mediated Suppression in the Context of Autoimmunity. *J Immunol* 2015; **195**: 528-540.
  15. Walter GJ, Fleskens V, Frederiksen KS, Rajasekhar M, Menon B, Gerwien JG *et al.* Phenotypic, functional, and gene expression profiling of peripheral CD45RA<sup>+</sup>

- and CD45RO+ CD4+CD25+CD127(low) Treg cells in patients with chronic rheumatoid arthritis. *Arthritis Rheumatol* 2016; **68**:103-116.
16. Buchwald ZS, Kiesel JR, DiPaolo R, Pagadala MS, Aurora R. Osteoclast activated FoxP3+ CD8+ T-cells suppress bone resorption in vitro. *PLoS One* 2012; **7**: e38199.
  17. Roato I, Caldo D, D'Amico L, D'Amelio P, Godio L, Patanè S *et al.* Osteoclastogenesis in peripheral blood mononuclear cell cultures of periprosthetic osteolysis patients and the phenotype of T cells localized in periprosthetic tissues. *Biomaterials* 2010; **31**: 7519-7525.
  18. Roark CL, French JD, Taylor MA, Bendele AM, Born WK, O'Brien RL. Exacerbation of collagen-induced arthritis by oligoclonal, IL-17-producing gamma delta T cells. *J Immunol* 2007; **179**: 5576-5583.
  19. Pöllinger B, Junt T, Metzler B, Walker UA, Tyndall A, Allard C *et al.* Th17 cells, not IL-17+  $\gamma\delta$  T cells, drive arthritic bone destruction in mice and humans. *J Immunol* 2011; **186**: 2602-2612.
  20. Ito Y, Usui T, Kobayashi S, Iguchi-Hashimoto M, Ito H, Yoshitomi H *et al.* Gamma/delta T cells are the predominant source of interleukin-17 in affected joints in collagen-induced arthritis, but not in rheumatoid arthritis. *Arthritis Rheum* 2009; **60**: 2294-2303.
  21. Pappalardo A, Thompson K. Novel immunostimulatory effects of osteoclasts and macrophages on human  $\gamma\delta$  T cells. *Bone* 2015; **71**:180-188.
  22. Pappalardo A, Thompson K. Activated  $\gamma\delta$  T cells inhibit osteoclast differentiation and resorptive activity in vitro. *Clin Exp Immunol* 2013; **174**:281-291.
  23. Benasciutti E, Mariani E, Oliva L, Scolari M, Perilli E, Barras E *et al.* MHC class II transactivator is an in vivo regulator of osteoclast differentiation and bone

- homeostasis co-opted from adaptive immunity. *J Bone Miner Res* 2014; **29**: 290-303.
24. Bozec A, Zaiss MM, Kagwiria R, Voll R, Rauh M, Chen Z *et al*. T cell costimulation molecules CD80/86 inhibit osteoclast differentiation by inducing the IDO/tryptophan pathway. *Sci Transl Med* 2014; **6**:235ra60.
25. Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E *et al*. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest* 2012; **122**:1791-1802.
26. Krishnamurthy A, Joshua V, Haj Hensvold A, Jin T, Sun M, Vivar N *et al*. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. *Ann Rheum Dis* 2016; **75**:721-729.
27. Hensvold AH, Joshua V, Li W, Larkin M, Qureshi F, Israelsson L *et al*. Serum RANKL levels associate with anti- citrullinated protein antibodies in early untreated rheumatoid arthritis and are modulated following methotrexate. *Arthritis Res Ther* 2015; **17**: 239.
28. Haugeberg G, Lodder MC, Lems WF, Uhlig T, Ørstavik RE, Dijkmans BA *et al*. Hand cortical bone mass and its associations with radiographic joint damage and fractures in 50-70 year old female patients with rheumatoid arthritis: cross sectional Oslo-Truro-Amsterdam (OSTRA) collaborative study. *Ann Rheum Dis* 2004; **63**: 1331-1334.
29. Charatcharoenwitthaya N, Khosla S, Atkinson EJ, McCready LK, Riggs BL. Effect of blockade of TNF-alpha and interleukin-1 action on bone resorption in early postmenopausal women. *J Bone Miner Res* 2007; **22**: 724-729.
30. Klimek E, Skalska A, Kwaśny-Krochin B, Surdacki A, Sulicka J, Korkosz M *et al*. Differential associations of inflammatory and endothelial biomarkers with disease

activity in rheumatoid arthritis of short duration. *Mediators Inflamm* 2014; **2014**: 681635.

31. Manolagas SC. Wnt signaling and osteoporosis. *Maturitas* 2014; **78**: 233-237.
32. Wang SY, Liu YY, Ye H, Guo JP, Li R, Liu X *et al*. Circulating Dickkopf-1 is correlated with bone erosion and inflammation in rheumatoid arthritis. *J Rheumatol* 2011; **38**: 821-827.
33. Garnero P, Tabassi NC, Voorzanger-Rousselot N. Circulating dickkopf-1 and radiological progression in patients with early rheumatoid arthritis treated with etanercept. *J Rheumatol* 2008; **35**: 2313-2315.
34. Anginot A, Dacquin R, Mazzorana M, Jurdic P. Lymphocytes and the Dap12 adaptor are key regulators of osteoclast activation associated with gonadal failure. *PLoS One* 2007; **2**: e585.
35. Lee SK, Kadono Y, Okada F, Jacquin C, Koczon-Jaremko B, Gronowicz G *et al*. T lymphocyte-deficient mice lose trabecular bone mass with ovariectomy. *J Bone Miner Res* 2006; **21**:1704-1712.
36. Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao Y, Qian WP *et al*. Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-gamma-induced class II transactivator. *Proc Natl Acad Sci USA* 2003; **100**: 10405-10410.
37. Eghbali-Fatourehchi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL, Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J Clin Invest* 2003; **111**: 1221-1230.

38. Li JY, Tawfeek H, Bedi B, Yang X, Adams J, Gao KY *et al.* Ovariectomy disregulates osteoblast and osteoclast formation through the T-cell receptor CD40 ligand. *Proc Natl Acad Sci USA* 2011; **108**: 768-773.
39. Rogers A, Eastell R. The effect of 17beta-estradiol on production of cytokines in cultures of peripheral blood. *Bone* 2001; **29**: 30-34.
40. Swanberg M, McGuigan FE, Ivaska KK, Gerdhem P, Åkesson K. Polymorphisms in the inflammatory genes CIITA, CLEC16A and IFNG influence BMD, bone loss and fracture in elderly women. *PLoS One* 2012; **7**: e47964.
41. Priyanka HP, Sharma U, Gopinath S, Sharma V, Hima L, ThyagaRajan S. Menstrual cycle and reproductive aging alters immune reactivity, NGF expression, antioxidant enzyme activities, and intracellular signaling pathways in the peripheral blood mononuclear cells of healthy women. *Brain Behav Immun* 2013; **32**: 131-143.
42. Javadian A, Salehi E, Bidad K, Sahraian MA, Izad M. Effect of estrogen on Th1, Th2 and Th17 cytokines production by proteolipid protein and PHA activated peripheral blood mononuclear cells isolated from multiple sclerosis patients. *Arch Med Res* 2014; **45**: 177-182.
43. Adeel S, Singh K, Vydareny KH, Kumari M, Shah E, Weitzmann MN *et al.* Bone loss in surgically ovariectomized premenopausal women is associated with T lymphocyte activation and thymic hypertrophy. *J Investig Med* 2013; **61**: 1178-1183.



44. Rodriguez-Garcia M, Biswas N, Patel MV, Barr FD, Crist SG, Ochsenbauer C *et al.* Estradiol reduces susceptibility of CD4+ T cells and macrophages to HIV-infection. *PLoS One* 2013; **8**: e62069.
45. Chen RY, Fan YM, Zhang Q, Liu S, Li Q, Ke GL *et al.* Estradiol inhibits Th17 cell differentiation through inhibition of ROR $\gamma$ T transcription by recruiting the ER $\alpha$ /REA complex to estrogen response elements of the ROR $\gamma$ T promoter. *J Immunol* 2015; **194**: 4019-4028.
46. Li JY, D'Amelio P, Robinson J, Walker LD, Vaccaro C, Luo T *et al.* IL-17A Is Increased in Humans with Primary Hyperparathyroidism and Mediates PTH-Induced Bone Loss in Mice. *Cell Metab* 2015; **22**: 799-810.
47. Li Y, Li A, Yang X, Weitzmann MN. Ovariectomy-induced bone loss occurs independently of B cells. *J Cell Biochem* 2007; **100**: 1370-1375.
48. Yeo L, Toellner KM, Salmon M, Filer A, Buckley CD, Raza K *et al.* Cytokine mRNA profiling identifies B cells as a major source of RANKL in rheumatoid arthritis. *Ann Rheum Dis* 2011; **70**: 2022-2028.
49. Kanematsu M, Sato T, Takai H, Watanabe K, Ikeda K, Yamada Y. Prostaglandin E2 induces expression of receptor activator of nuclear factor-kappa B ligand/osteoprotegerin ligand on pre-B cells: implications for accelerated osteoclastogenesis in estrogen deficiency. *J Bone Miner Res* 2000; **15**: 1321-1329.
50. Onal M, Xiong J, Chen X, Thostenson JD, Almeida M, Manolagas SC *et al.* Receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) protein expression by B

lymphocytes contributes to ovariectomy-induced bone loss. *J Biol Chem* 2012; **287**: 29851-29860.

51. Breuil V, Ticchioni M, Testa J, Roux CH, Ferrari P, Breitmayer JP *et al.* Immune changes in post-menopausal osteoporosis: the Immunos study. *Osteoporos Int* 2010; **21**: 805-814.

52. Wheeler G, Hogan VE, Teng YK, Tekstra J, Lafeber FP, Huizinga TW *et al.* Suppression of bone turnover by B-cell depletion in patients with rheumatoid arthritis. *Osteoporos Int* 2011; **22**: 3067-3072.

53. Abe T, AlSarhan M, Benakanakere MR, Maekawa T, Kinane DF, Cancro MP *et al.* The B Cell-Stimulatory Cytokines BLyS and APRIL Are Elevated in Human Periodontitis and Are Required for B Cell-Dependent Bone Loss in Experimental Murine Periodontitis. *J Immunol* 2015; **195**: 1427-1435.

54. Peterson CT, Sharma V, Elmén L, Peterson SN. Immune homeostasis, dysbiosis and therapeutic modulation of the gut microbiota. *Clin Exp Immunol* 2015; **179**: 363-377.

55. Ohlsson C, Engdahl C, Fåk F, Andersson A, Windahl SH, Farman HH *et al.* Probiotics protect mice from ovariectomy-induced cortical bone loss. *PLoS One* 2014; **9**: e92368.

56. Parvaneh K, Ebrahimi M, Sabran MR, Karimi G, Hwei AN, Abdul-Majeed S *et al.* Probiotics (*Bifidobacterium longum*) Increase Bone Mass Density and Upregulate Sparc and Bmp-2 Genes in Rats with Bone Loss Resulting from Ovariectomy. *Biomed Res Int* 2015; **2015**: 897639.

57. Weaver CM, Martin BR, Nakatsu CH, Armstrong AP, Clavijo A, McCabe LD *et al.* Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation. *J Agric Food Chem* 2011; **59**: 6501-6510.
58. Jones ML, Martoni CJ, Prakash S. Oral supplementation with probiotic *L. reuteri* NCIMB 30242 increases mean circulating 25-hydroxyvitamin D: a post hoc analysis of a randomized controlled trial. *J Clin Endocrinol Metab* 2013; **98**: 2944-2951.
59. Whisner CM, Martin BR, Schoterman MH, Nakatsu CH, McCabe LD, McCabe GP *et al.* Galacto-oligosaccharides increase calcium absorption and gut bifidobacteria in young girls: a double-blind cross-over trial. *Br J Nutr* 2013; **110**: 1292-1303.
60. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G *et al.* A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am J Clin Nutr* 2005; **82**: 471-476.
61. Weaver CM. Diet, gut microbiome, and bone health. *Curr Osteoporos Rep* 2015; **13**: 125-130.